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In Vitro Effects of the Endocrine Disruptor p,p'DDT on Human Follitropin Receptor

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Short running title: FSHR as a target of p,p'DDT

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Abstract

Background: p,p'DDT is an environmental persistent endocrine disruptor (ED). Several studies have shown an association between p,p'DDT exposure and reproductive abnormalities.

Objectives: To investigate putative effects of p,p'DDT on the Follitropin receptor (FSHR) function.

Methods and Results: We investigated the impact of p,p'DDT on the FSHR activity and its interaction with the receptor, using Chinese Hamster Ovary (CHO) cells stably expressing the human FSHR. p,p'DDT, at 5 μM, increased the maximal response of FSHR to follitropin by 32 ± 7.45 %.Otherwise, 5μM p,p'DDT decreased the basal activity and didn't influence the maximal response of the closely related LH/hCG receptor to human chorionic gonadotropin hormone. The potentiating effect of p,p'DDT was specific of FSHR. Moreover, in cells, that didn't express FSHR, the p,p'DDT didn't have effect on cAMP response. So, the potenting effect of p,p'DDT was dependent on FSHR. In addition, p,p'DDT increased the sensitivity of FSHR to hCG and to a low molecular weight agonist (16a) of FSHR. Basal activity in response to p,p'DDT and potentiation of the FSHR response to FSH by p,p'DDT varied among FSHR mutants with altered transmembrane domains (TMD), consistent with an effect of p,p'DDT via TMD binding. This was corroborated by the results of docking p,p'DDT and 16a into the FSHR transmembrane bundle simultaneously.

Conclusion: p,p'DDT acted as a positive allosteric modulator of FSHR in our experimental model. These findings suggest that G Protein-coupled receptors are additional targets of endocrine disruptors.

Introduction

The health impact of endocrine disruptors (ED) is a growing concern as targets and effects on animals and humans are diverse, and the list of disruptors seems endless (Zoeller et al. 2012). Among ED, DDT, an organochlorine pesticide composed mainly of p,p'DDT, was largely used after the Second World War for its insecticide properties. Although it was banned in the seventies in the Western world, it is still used in developing countries. It is known to accumulate in fat tissue and to be highly persistent in the environment. Contamination of soil and water allows it to ascend the food chain and reach humans (Sudharshan et al. 2012). Children are also exposed to maternal p,p'DDT in utero and through breast feeding. For example, the average serum concentration of p,p'DDT approaches 4 ng/g ($7.3\ 10^{-11}$M) of body lipids in the French population (Saoudi et al. 2014). However, in a population of young men in South Africa, where DDT continues to be sprayed, the average lipid-adjust serum concentration of p,p'DDT reaches 90.23 μg/g ($1.5\ 10^{-6}$M) (Aneck-Hahn et al. 2007). According to epidemiological data, exposure to p,p'DDT is associated with decreased semen parameters (Jeng 2014; Martenies and Perry 2013). Moreover, cryptorchidism, hypospadias and micropenis have been reported to be associated with *in utero* exposure to p,p'DDT (Damgaard et al. 2006; Gaspari et al. 2012; Hosie et al. 2000; Jeng 2014; Rignell-Hydbom et al. 2012) and the concept of testicular dysgenesis syndrome has been proposed to encompass the spectrum of male reproductive outcomes that have been associated with ED exposure(Wohlfahrt-Veje et al. 2009). In addition, p,p'DDT has been measured in ovarian follicular fluids of women (Jarrell et al. 1993; Jirsová et al. 2010), and p,p'DDT exposures have been associated with evidence of reduced fertility (Jirsová et al. 2010; Venners et al.

2005).Shorter menstrual cycles (Windham et al. 2005), and reduced probability of pregnancy in daughters from women exposed to p,p'DDT (Cohn et al. 2003) have been reported. Moreover, serum p,p'DDT and *in utero* exposure has been associated with precocious puberty in girls (Ouyang et al. 2005; Vasiliu et al. 2004). Some *in vitro* studies have shown that p,p'DDT exhibits anti-androgenic and estrogen-like effects (Aubé et al. 2011; Kojima et al. 2004; Li et al. 2008; Schug et al. 2011; Strong et al. 2014; Wang et al. 2010) through binding to nuclear receptors. Gonadal function is under pituitary control *via* the gonadotropin hormones: Follicle stimulating hormone (FSH) and Luteinizing hormone (LH). A third hormone, chorionic gonadotropin hormone (hCG), is secreted by the placenta and controls the function of ovary during gestation, in primates.

The FSH receptor (FSHR) is a plasma membrane receptor belonging, as well as the LH/hCG receptor, to the G protein-coupled receptor (GPCR) superfamily (Minegishi et al. 1991). It is expressed in Sertoli and granulosa cells in male and female gonads, respectively, and is required for normal spermatogenesis on one hand, and growth and maturation of ovarian follicles, as well as estrogen production, on the other hand (Siegel et al. 2013). It is mainly coupled to the cAMP pathway through the Gs protein and Adenylyl Cyclase (AC) (Means et al. 1974; Minegishi et al. 1994). However, it can also couple to several other effectors such as Gq and βarrestin (Gloaguen 2011; Landomiel et al. 2014; Ulloa-Aguirre et al. 2007). Previously, p,p'DDT has been shown to disturb the downstream signaling of the FSHR (Bernard et al. 2007; Rossi et al. 2007), and p,p'DDE, a metabolite of p,p'DDT increases the FSH-induced progesterone production (Crellin et al. 1999) and aromatase activity (Younglai 2004) in porcine and human granulosa cells, respectively.

Although FSH interacts with the large extracellular N-terminal domain of its receptor, small sized molecules have been designed that are able to activate or inhibit the FSHR (Arey et al. 2008; Dias et al. 2011, 2014; Sriraman et al. 2014; van Koppen et al. 2013; Wrobel et al. 2006; Yanofsky et al. 2006; Yu et al. 2014). They bind to the transmembrane domain (TMD) of FSHR and can be considered as allosteric modulators. The p,p'DDT structure shows structural homologies with the one of some allosteric modulators of FSHR (Dias et al. 2011; van Koppen et al. 2013). This suggests that p,p'DDT may interact with allosteric sites on the FSHR. We investigated the effect of p,p'DDT in Chinese Hamster Ovary (CHO) cells stably transfected with the human FSHR (CHO-FSHR) and responsive to FSH. We show that p,p'DDT increases the cAMP response to FSH through an interaction with the TMD of the FSHR, providing evidence for an allosteric effect of p,p'DDT on this receptor.

Materials and Methods

Reagents

Chemicals: p,p'DDT, forskolin, IBMX, salmon calcitonin, p,p'DDE, o,p'DDT and Bisphenol A (BPA) were purchased from Sigma-Aldrich (St Louis, US), and dissolved in DMSO. The gonadotropin hormones hFSH (Gonal-f) and hCG (o'vitrelle) were purchased from Merck-Serono (Lyon, France). The conversion between International Units per milliliter and nanograms per milliliter or molar concentrations is 1 UI/ml recombinant hFSH corresponds to 100 ng/ml or 3.3 nM, and 1 UI/ml recombinant hCG corresponds to 62 ng/ml or 2 nM.

Plasmids: FSHR mutants T3.32A, T3.32I, H7.42A, T3.32I-H7.42A, and rat FSHR were kindly provided by Dr S. Costagliola. Amino acid residues are numbered according to the Ballesteros system (Sealfon et al. 1995).

Cell culture

CHO cell lines stably transfected with the human FSHR were previously described (Bonomi et al. 2006). CHO and CHO-FSHR cell lines were maintained in DMEM (PAA, Pasching, Austria) containing 10% fetal calf serum (FCS Biowest Nuaille, France), 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin (Lonza, Verviers, Belgium) at 37°C in a humidified incubator gassed with 5% CO2.

cAMP assay

cAMP production was determined using the Promega GloSensor cAMP assay (Promega, Fitchburg, USA), (Binkowski et al. 2011). Briefly, cells were seeded (20,000 cells/well) in white 96-well clear-bottomed microplates. The next day, cells were transfected with pGloSensorTM-22F cAMP plasmid (150 ng), encoding an engineered cAMP-sensitive luciferase, using Lipofectamine LTX (Invitrogen, Cergy-Pontoise, France) according to the manufacturer's instructions. Twenty-four hours after transfection, medium was removed, and cells were incubated for 2h at room temperature in 90 µL of the equilibration medium, a substrate-containing medium (GloSensor^{TM} cAMP reagent) diluted at 6% in DMEM containing 10% FCS. Cells were incubated with drugs for 30 min, and end-point luminescence was recorded on a Synergy^{TM} 2 microplate luminometer (Biotek,Vermont, USA). Graphs were fitted to data using GraphPad Prism 6 and results are expressed as the mean ± SEM from at least three independent experiments performed in triplicate. Concentration-response data were fitted using a four-parameter equation.

Molecular modeling and Induced fit docking

FSHR was modeled from I1.29 to S7.69 with MODELLER 9v8 (Sali and Blundell 1993) by homology with rhodopsin (PDB code 3C9L), except TM5 that was modeled as a straight helix (Kleinau et al. 2011). The FSHR model was prepared for docking using the Protein Preparation Wizard in the Schrödinger Suite 2012 (Schrödinger Suite 2012 Protein Preparation Wizard; Epik version 2.3, Impact version 5.8, Prime version 3.1). Protonation states were assigned for all titrable groups according to pH 7 using Propka (Olsson et al. 2011) and the model was then energy minimized using the OPLS2005 force field with a restraint in which the maximum heavy atom RMSD was set to 0.30 Å. The induced fit dockings (IFDs) (Sherman et al. 2006a, 2006b) were performed in the Schrödinger Suite 2012 (Schrödinger Suite 2012 Induced Fit Docking Protocol; Glide version 5.8; Prime version 3.1) according to a three-step protocol: (1) the initial Glide docking was performed with 0.5 scaling of all van der Waals radii for a maximum of 50 poses; (2) side chains of residues within 5 Å of the ligand were optimized, with an implicit membrane model; (3) a final Glide docking was performed for complexes that were within 30 kcal/mol of the best scoring complex and within the top 20 overall. p,p-DDT was docked into the minor pocket (Hoyer et al. 2013) (TM1-3,7). The pose with the best IFD score was then used as input for an IFD calculation for 16a in the major site. Additionally, 16a was docked into the major pocket (TM3-7) with the minor site unoccupied, and the highest scoring pose was used as input in an IFD calculation for p,p-DDT in the minor site. The reverse procedure, docking of 16a in the major pocket then binding of p,p'DDT in the minor pocket, led to similar results as first binding p,p'DDT and then binding 16a (data not shown).

Statistical analyses

Results represent mean ± SEM of at least 9 samples, obtained in at least 3 independent experiments for each condition. Statistical analyses were performed using non-parametric Mann-Whitney test (Prism 6, GraphPad Software, Inc., San Diego, CA).

Results

Effect of p,p'DDT on FSH-dependent cAMP production

To investigate the effect of p,p'DDT on FSHR, we used Chinese Hamster Ovary (CHO) cell lines stably transfected with the human FSHR (CHO-FSHR) (Bonomi et al. 2006). We first verified that 5.10^{-6}M p,p'DDT did not induce cell death (see Figure S1). The dose-response curve of hFSH in these cells indicated an EC_{50} value of 0.03 ± 0.002 UI/mL (data not shown). p,p'DDT enhanced the cAMP accumulation induced by two different doses of hFSH 3.10^{-2} UI/mL or 3 UI/mL in coincubation (Figure 1A) up to 157 ± 10.57 % of maximal response. We next examined the effect of the most potent concentration of p,p'DDT (5.10^{-6} M) on the FSH dose-response curve. The maximal response was increased by 32 ± 7.45 % (8 experiments) whereas the EC_{50} was unaffected (0.02UI/mL vs 0.03UI/mL) (Figure 1B). In contrast to the increase of the maximal response, there was no impact on the basal activity of the FSHR (Figure 1C). In kinetic study the effect of p,p'DDT was detected as early as 6 min (Figure 1D), whereas the maximal response to FSH with or without p,p'DDT was reached at 13 min and 12 min, respectively (Figure 1D).

Effects of p,p'DDT on other receptors

In CHO-FSHR cells, cAMP production in response to calcitonin stimulation of the endogenously expressed calcitonin receptor was not affected by co-incubation with

p,p'DDT (Figure 2A). p,p'DDT also did not induce cAMP in response to calcitonin in CHO cells (data not shown).

The effect of p,p'DDT on LH/hCG receptor (LH/hCGR), a closely related receptor belonging to the same family as FSHR (Vassart et al. 2004), was also analyzed. In CHO cell lines stably transfected with the human LH/hCGR (CHO-LH/hCGR) (Bonomi et al. 2006). p,p'DDT decreased the cAMP production stimulated by hCG at 10^{-2} UI/mL, in a dose dependent manner, down to 80 ± 3.7% of the response in absence of p,p'DDT (Figure 2B). The response to hCG at 100 UI/mL also was increased in response to p,p'DDT, but the increase was significant only at the lowest p,p'DDT dose (10^{-7}M) (Figure 2B). Interestingly, p,p'DDT decreased the basal activity of LH/hCGR by 50 ± 9%.

To examine the putative impact of p,p'DDT on the downstream effectors of FSHR, we first tested its effects on forskolin-induced cAMP accumulation in CHO-FSHR and CHO cells (Figure 2C). There was a dose dependent increase of the response to forskolin in CHO-FSHR cells reaching 140 ± 6.71% of the control value. This effect was not observed in CHO cells (Figure 2C). In addition, we did not observe an effect of p,p'DDT on the response to forskolin in HEK293 cells or in the CHO-LH/hCGR cell line (data not shown). These findings suggest that the effect of p,p'DDT on AC requires the presence of FSHR. We also analyzed the effects of p,p'DDT on phosphodiesterases (PDE) activity. p,p'DDT further increased the higher FSH stimulated cAMP production observed in presence of IBMX, a PDE inhibitor (Figure 2D).

Interactions between p,p'DDT and the FSH receptor transmembrane domain

The low molecular weight agonist (LMW), 16a (kindly provided by Dr Wrobel, see Figure S2), (Wrobel et al. 2006) is able to stimulate the FSHR with the same efficiency as FSH through binding to the TMD (Yanofsky et al. 2006). As shown in figure 3A, increasing concentrations of p,p'DDT potentiate the response to 16a with a 10-fold decrease in the 16a EC50 in presence of 10^{-5}M p,p'DDT.

To analyze putative interactions between p,p'DDT and the TMD, several mutants in helix 3 and in helix 7: T3.32A, T3.32I, H7.42A, T3.32I-H7.42A were used. The mutations T3.32I and T3.32A have been identified in women with spontaneous ovarian hyperstimulation syndrome (Montanelli et al. 2004a; Vasseur et al. 2003), they increase the basal activity of the receptor and decrease its ligand specificity (Montanelli et al. 2004b). T3.32, highly conserved in the glycoprotein hormone receptors, is located in the cavity formed by the TMD and can interact with the histidine residue at position 7.42 (Montanelli et al. 2004b). The mutants were properly expressed at the cell surface and responsiveness to FSH was unaffected (see Figure S3). While substitution of T3.32 by alanine maintained the potentiating activity of p,p'DDT on the maximal response induced by FSH (Figure 3B), its substitution by isoleucine abolished this effect (Figure 3C). In addition, p,p'DDT reduced by 30 ± 0.06 % the basal activity of the mutant T3.32I. The substitution of H7.42 by an alanine reversed the potentiating effect of p,p'DDT into a 20 ± 6.28 % inhibition (Figure 3D). The basal activity of FSHR H7.42A was unaffected by p,p'DDT. The double mutant T3.32I-H7.42A did not display any sensitivity to p,p'DDT either on the maximal response or on the basal activity (Figure 3E). Finally, we evaluated the effect of p,p'DDT on the rat FSHR, transiently expressed in CHO cells line. As

shown in Figure 3F, an approximately 1.40-fold increase of maximal response without any modification of the EC50 (0.04 UI/mL vs 0.05 UI/mL) was observed. In contrast to hFSHR p,p'DDT induced a significant reduction of the basal activity (30 ± 0.09%) of the rat receptor.

The allosteric effect of p,p'DDT on the activation of FSHR by 16a strongly suggests that both molecules can bind to FSHR. Preliminary models indicated that binding of both molecules within the transmembrane cavity required p,p'DDT and 16a in the minor and major binding pockets, respectively (Rosenkilde et al. 2010). The three best-scoring docking poses of p,p'DDT in the minor pocket position showed that one of the *p*-chlorophenyl groups was located in vicinity of Thr3.32 and His7.42 (Figure 3G). This was consistent with the effects of the mutation of these residues.

Effects of p,p'DDT on the specificity of the FSH receptor

Because some activating mutations T3.32I of the FSHR TMD make it more responsive to hCG, (De Leener et al. 2006; Montanelli et al. 2004a, 2004b; Smits et al. 2003; Ulloa-Aguirre et al. 2014; Vasseur et al. 2003), the effect of p,p'DDT on the specificity of the FSHR was also analyzed. p,p'DDT enhanced the FSHR response to increasing concentration of hCG but did not alter the sensitivity of FSHR to thyrotropin (Figure 4), in contrast to the effect of some mutations T3.32A, T3.32I, H7.42A, T3.32I-H7.42A (Montanelli et al. 2004b; Vasseur et al. 2003).

Effect of p,p'DDT related molecules on the FSHR

p,p'DDT has a biphenolic structure. We hypothesized that other chemicals structurally related to p,p'DDT could have similar effects on the FSH-induced cAMP response. p,p'DDT, its metabolite p,p'DDE and o,p'DDT diverge by the number or the position of

the chlorine atoms. BPA harbors OH groups instead of chlorine atoms (see Figure S2). The dose-response relations of p,p'DDE were non-monotonic on the cAMP accumulation induced by two different doses of hFSH 3.10^{-2} UI/mL or 3 UI/mL. The strongest effects, an increase of 66 and 34%, were obtained for 10^{-6}M p,p'DDE (Figure 5A). For o,p'DDT there was no significant effect on the response to 3.10^{-2} UI/mL hFSH, while the response to 3 UI/mL hFSH increased of 25% at 10^{-7}M o,p'DDT, and was not significant at 10^{-5}M (Figure 5B). Finally, 10^{-5}M BPA decreased by 30 and 15 % the cAMP production stimulated by FSH 3.10^{-1}UI/ml and 3UI/ml, respectively (Figure 5C). We also verified that p,p'DDE, o,p'DDT and BPA do not induce cell death (see Figure S1).

Discussion

In the present work, we examined the FSHR as a putative target of p,p'DDT, a known disruptor of reproductive function (Bergman et al. 2013). Previous studies (Bernard et al. 2007; Crellin et al. 1999; Younglai 2004) had shown an alteration of the response of gonadal cells to FSH. Because different molecules and pathways can be affected by p,p'DDT, it was necessary to isolate the FSHR from its native environment, namely sertoli or granulosa cells, to specifically identify disruption of its functions. Therefore the human FSHR was overexpressed in CHO cells.

We showed that p,p'DDT potentiates the maximal FSH stimulated cAMP production by the FSHR and thus acts as a positive allosteric modulator. The kinetic of response to FSH indicates that p,p'DDT acts on the early steps of activation of the FSHR rather than on extinction /prolongation of the signal. Indeed, the effect of p,p'DDT was obvious within 6 minutes (Figure 1D). Several facts argue for a direct effect of p,p'DDT on the FSHR. The effect of p,p'DDT required the presence of FSHR because there was no

increase in basal nor calcitonin-stimulated cAMP production in untransfected CHO cells. The effect was specific of FSHR because the closely related LH/hCGR responded differently than the FSHR, with a decrease in the basal activity, and no high potentiation of the maximal response. 10^{-7}M p,p'DDT increased by 8% the LH/hCGR maximal response *versus* 32% for 5.10^{-6}M p,p'DDT on the FSHR maximal response.

Although the experiments aimed at studying the effect of p,p'DDT on FSHR, off target effects cannot be excluded as the FSHR was studied in a cellular environment. Thus, putative actions on the PDE, AC or G protein were examined. The increase in cAMP concentration was not due to the inhibition of PDEs, because p,p'DDT still enhanced cAMP after inhibition of PDEs by IBMX. The forskolin induced cAMP production was potentiated only in the presence of the FSHR. This may be an indication of an effect of p,p'DDT on the FSHR even in absence of FSH. Although no increase in the basal activity of the receptor could be detected, this potentiation of the response to forskolin is reminiscent of the effect observed when studying constitutively active mutant GPCRs, and is interpreted as an indication of a "pre-activated" state of the GPCR and of the G protein (Alewijnse et al. 1997). Interestingly, the potentiation of the response to forskolin was not due only to the expression of FSHR but required the presence of p,p'DDT (see Figure S4). Therefore, it can be hypothesized that p,p'DDT binding induces a pre-coupling of FSHR with Gs, facilitating the activation of the AC. This will require further investigations.

Several chemicals related to p,p'DDT (p,p'DDE, o,p'DDT and BPA) also affected the FSHR, though differently, confirming the specificity of the p,p'DDT effect. In addition, some mutations of the FSHR in the TMD abolished the effect of p,p'DDT, while

preserving the response to FSH. This suggests that a binding site for the disruptor is located in the TMD.

The electrostatic interactions between ligand and receptor binding pocket play a crucial role in agonist or inverse agonist action (Vezzi et al. 2013). Our results suggest that the chlorine atoms are decisive for the potentiating effect of p,p'DDT on FSHR. This is further illustrated by the inhibiting effect of the 10^{-5}M chlorideless BPA.

The positive modulation by p,p'DDT is also observed when the FSHR is stimulated by 16a, indicating that both molecules can interact simultaneously with the receptor, which has been corroborated by molecular docking, with p,p'DDT and 16a in the minor and major binding pockets, respectively. The preferred binding pose of p,p'DDT in the minor pocket is consistent with the observed effects of mutation of Thr3.32 and His7.42. The switch from positive to negative allosteric modulation by p,p'DDT upon the H7.42A mutation is reminiscent of a LMW ligand of thyrotropin receptor whose antagonist effect was reversed to agonist upon a point mutation (Hoyer et al. 2013).

The binding of p,p'DDT to the TMD supposedly modifies the physicochemical environment of the transmembrane helices of the receptor. This modifies the free energy landscape of the receptor, leading to p,p'DDT acting as a positive allosteric modulator. Also the ectodomain of the receptor is proposed to behave as an inhibitor of the TMD (Jiang et al. 2012). The binding of p,p'DDT may participate also to the release of this inhibitory interaction. This may explain the enhanced response to hCG as well.

Other mechanisms may also participate in the allosteric modulation of the FSHR response by p,p'DDT, such as an effect on its internalization and desensitization (Krishnamurthy et al. 2003). The rapid kinetics of the p,p'DDT effect does not make this

likely. The receptor oligomerization (Jiang et al. 2014) may also be affected. Further studies will be necessary to fully understand the mechanisms of allosteric effect of p,p'DDT. Morover, p,p'DDT can potentially stabilize different conformations thereby leading to biased agonism as described for the FSHR LMW agonists (Landomiel et al. 2014). It will be interesting to study the impact of p,p'DDT on the others signaling pathways.

Several studies indicate that an increased activity of the FSH/FSHR pathway (Kumar et al. 1999; Peltoketo et al. 2010), including illegitimate stimulation by hCG (Montanelli et al. 2004a; Smits et al. 2003; Vasseur et al. 2003), may result in adverse effects on reproduction and sexual development. The increased response to FSH in presence of p,p'DDT we show *in vitro*, and the gain of sensitivity to hCG (and presumably LH), may therefore be deleterious *in vivo*. Increased stimulation due to EDs may contribute to some cases of unexpected and unexplained spontaneous ovarian hyperstimulation syndrome occurring during controlled ovarian stimulation by gonadotropins in assisted reproduction procedures (Jirsová et al. 2010; Machtinger and Orvieto 2014). Whether an *in utero* illegitimate stimulation of FSHR by hCG can worsen the male and female fetal gonad damage related to p,p'DDT exposure is not known. Our finding that, *in vitro*, p,p'DDT reduced basal activity in the rat FSHR while increasing activity in the human FSHR, raises concerns about extrapolating implications of *in vivo* findings from animal models to human health.

In conclusion, our *in vitro* findings suggest that the human FSHR is a target for p,p'DDT, and they support the potential for effects of p,p'DDT and other endocrine disruptors on other GPCRs.

References

Alewijnse AE, Smit MJ, Rodriguez Pena MS, Verzijl D, Timmerman H, Leurs R. 1997. Modulation of forskolin-mediated adenylyl cyclase activation by constitutively active G(S)-coupled receptors. FEBS Lett. 419: 171–174.

Aneck-Hahn NH, Schulenburg GW, Bornman MS, Farias P, de Jager C. 2007. Impaired semen quality associated with environmental DDT exposure in young men living in a malaria area in the Limpopo Province, South Africa. J. Androl. 28:423–434; doi:10.2164/jandrol.106.001701.

Arey BJ, Yanofsky SD, Claudia Pérez M, Holmes CP, Wrobel J, Gopalsamy A, et al. 2008. Differing pharmacological activities of thiazolidinone analogs at the FSH receptor. Biochem. Biophys. Res. Commun. 368:723–728; doi:10.1016/j.bbrc.2008.01.119.

Aubé M, Larochelle C, Ayotte P. 2011. Differential effects of a complex organochlorine mixture on the proliferation of breast cancer cell lines. Environ. Res. 111:337–347; doi:10.1016/j.envres.2011.01.010.

Bergman \AAke, Heindel JJ, Jobling S, Kidd KA, Zoeller RT. 2013. Endocrine Disrupting Chemicals-2012.

Bernard L, Martinat N, Lécureuil C, Crépieux P, Reiter E, Tilloy-Ellul A, et al. 2007. Dichlorodiphenyltrichloroethane impairs follicle-stimulating hormone receptor-mediated signaling in rat Sertoli cells. Reprod. Toxicol. 23:158–164; doi:10.1016/j.reprotox.2006.11.002.

Binkowski BF, Fan F, Wood KV. 2011. Luminescent biosensors for real-time monitoring of intracellular cAMP. Methods Mol. Biol. Clifton NJ 756:263–271; doi:10.1007/978-1-61779-160-4_14.

Bonomi M, Busnelli M, Persani L, Vassart G, Costagliola S. 2006. Structural differences in the hinge region of the glycoprotein hormone receptors: evidence from the sulfated tyrosine residues. Mol. Endocrinol. Baltim. Md 20:3351–3363; doi:10.1210/me.2005-0521.

Cohn BA, Cirillo PM, Wolff MS, Schwingl PJ, Cohen RD, Sholtz RI, et al. 2003. DDT and DDE exposure in mothers and time to pregnancy in daughters. Lancet 361:2205–2206; doi:10.1016/S0140-6736(03)13776-2.

Crellin NK, Rodway MR, Swan CL, Gillio-Meina C, Chedrese PJ. 1999. Dichlorodiphenyldichloroethylene potentiates the effect of protein kinase A pathway activators on progesterone synthesis in cultured porcine granulosa cells. Biol. Reprod. 61: 1099–1103.

Damgaard IN, Skakkebaek NE, Toppari J, Virtanen HE, Shen H, Schramm K-W, et al. 2006. Persistent pesticides in human breast milk and cryptorchidism. Environ. Health Perspect. 114: 1133–1138.

De Leener A, Montanelli L, Van Durme J, Chae H, Smits G, Vassart G, et al. 2006. Presence and Absence of Follicle-Stimulating Hormone Receptor Mutations Provide Some Insights into Spontaneous Ovarian Hyperstimulation Syndrome Physiopathology. J. Clin. Endocrinol. Metab. 91:555–562; doi:10.1210/jc.2005-1580.

Dias JA, Bonnet B, Weaver BA, Watts J, Kluetzman K, Thomas RM, et al. 2011. A negative allosteric modulator demonstrates biased antagonism of the follicle stimulating hormone receptor. Mol. Cell. Endocrinol. 333:143–150; doi:10.1016/j.mce.2010.12.023.

Dias JA, Campo B, Weaver BA, Watts J, Kluetzman K, Thomas RM, et al. 2014. Inhibition of follicle-stimulating hormone-induced preovulatory follicles in rats treated with a nonsteroidal negative allosteric modulator of follicle-stimulating hormone receptor. Biol. Reprod. 90:19; doi:10.1095/biolreprod.113.109397.

Gaspari L, Sampaio DR, Paris F, Audran F, Orsini M, Neto JB, et al. 2012. High prevalence of micropenis in 2710 male newborns from an intensive-use pesticide area of Northeastern Brazil: Micropenis and endocrine-disrupting chemicals. Int. J. Androl. 35:253–264; doi:10.1111/j.1365-2605.2011.01241.x.

Gloaguen P. 2011. Mapping the follicle-stimulating hormone-induced signaling networks. Front. Endocrinol. 2; doi:10.3389/fendo.2011.00045.

Hosie S, Loff S, Witt K, Niessen K, Waag KL. 2000. Is there a correlation between organochlorine compounds and undescended testes? Eur. J. Pediatr. Surg. Off. J. Austrian Assoc. Pediatr. Surg. Al Z. Für Kinderchir. 10:304–309; doi:10.1055/s-2008-1072381.

Hoyer I, Haas A-K, Kreuchwig A, Schülein R, Krause G. 2013. Molecular sampling of the allosteric binding pocket of the TSH receptor provides discriminative pharmacophores for antagonist and agonists. Biochem. Soc. Trans. 41:213–217; doi:10.1042/BST20120319.

Jarrell JF, Villeneuve D, Franklin C, Bartlett S, Wrixon W, Kohut J, et al. 1993. Contamination of human ovarian follicular fluid and serum by chlorinated organic compounds in three Canadian cities. CMAJ Can. Med. Assoc. J. J. Assoc. Medicale Can. 148: 1321–1327.

Jeng HA. 2014. Exposure to endocrine disrupting chemicals and male reproductive health. Front. Public Health 2:55; doi:10.3389/fpubh.2014.00055.

Jiang X, Fischer D, Chen X, McKenna SD, Liu H, Sriraman V, et al. 2014. Evidence for Follicle-stimulating Hormone Receptor as a Functional Trimer. J. Biol. Chem. 289:14273–14282; doi:10.1074/jbc.M114.549592.

Jiang X, Liu H, Chen X, Chen P-H, Fischer D, Sriraman V, et al. 2012. Structure of follicle-stimulating hormone in complex with the entire ectodomain of its receptor. Proc. Natl. Acad. Sci. U. S. A. 109:12491–12496; doi:10.1073/pnas.1206643109.

Jirsová S, Masata J, Jech L, Zvárová J. 2010. Effect of polychlorinated biphenyls (PCBs) and 1,1,1-trichloro-2,2,-bis (4-chlorophenyl)-ethane (DDT) in follicular fluid on the results of in vitro fertilization-embryo transfer (IVF-ET) programs. Fertil. Steril. 93:1831–1836; doi:10.1016/j.fertnstert.2008.12.063.

Kleinau G, Hoyer I, Kreuchwig A, Haas A-K, Rutz C, Furkert J, et al. 2011. From molecular details of the interplay between transmembrane helices of the thyrotropin receptor to general aspects of signal transduction in family a G-protein-coupled receptors (GPCRs). J. Biol. Chem. 286:25859–25871; doi:10.1074/jbc.M110.196980.

Kojima H, Katsura E, Takeuchi S, Niiyama K, Kobayashi K. 2004. Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells. Environ. Health Perspect. 112: 524–531.

Krishnamurthy H, Kishi H, Shi M, Galet C, Bhaskaran RS, Hirakawa T, et al. 2003. Postendocytotic trafficking of the follicle-stimulating hormone (FSH)-FSH receptor complex. Mol. Endocrinol. Baltim. Md 17:2162–2176; doi:10.1210/me.2003-0118.

Kumar TR, Palapattu G, Wang P, Woodruff TK, Boime I, Byrne MC, et al. 1999. Transgenic models to study gonadotropin function: the role of follicle-stimulating hormone in gonadal growth and tumorigenesis. Mol. Endocrinol. 13: 851–865.

Landomiel F, Gallay N, Jégot G, Tranchant T, Durand G, Bourquard T, et al. 2014. Biased signalling in follicle stimulating hormone action. Mol. Cell. Endocrinol. 382:452–459; doi:10.1016/j.mce.2013.09.035.

Li J, Li N, Ma M, Giesy J, Wang Z. 2008. In vitro profiling of the endocrine disrupting potency of organochlorine pesticides. Toxicol. Lett.; doi:10.1016/j.toxlet.2008.10.002.

Machtinger R, Orvieto R. 2014. Bisphenol A, oocyte maturation, implantation, and IVF outcome: review of animal and human data. Reprod. Biomed. Online 29:404–410; doi:10.1016/j.rbmo.2014.06.013.

Mahalingaiah S, Missmer SA, Maity A, Williams PL, Meeker JD, Berry K, et al. 2012. Association of hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane (DDT), and dichlorodiphenyldichloroethylene (DDE) with in vitro fertilization (IVF) outcomes. Environ. Health Perspect. 120:316–320; doi:10.1289/ehp.1103696.

Martenies SE, Perry MJ. 2013. Environmental and occupational pesticide exposure and human sperm parameters: A systematic review. Toxicology 307:66–73; doi:10.1016/j.tox.2013.02.005.

Means AR, MacDougall E, Soderling TR, Corbin JD. 1974. Testicular Adenosine 3' : 5'-Monophosphate-dependent Protein Kinase REGULATION BY FOLLICLE-STIMULATING HORMONE. J. Biol. Chem. 249: 1231–1238.

Minegishi T, Igarashi S, Nakamura K, Nakamura M, Tano M, Shinozaki H, et al. 1994. Functional expression of the recombinant human FSH receptor. J. Endocrinol. 141: 369–375.

Minegishi T, Nakamura K, Takakura Y, Ibuki Y, Igarashi M, Minegish T [corrected to Minegishi T. 1991. Cloning and sequencing of human FSH receptor cDNA. Biochem. Biophys. Res. Commun. 175: 1125–1130.

Montanelli L, Delbaere A, Di Carlo C, Nappi C, Smits G, Vassart G, et al. 2004a. A mutation in the follicle-stimulating hormone receptor as a cause of familial spontaneous ovarian hyperstimulation syndrome. J. Clin. Endocrinol. Metab. 89: 1255–1258.

Montanelli L, Van Durme JJJ, Smits G, Bonomi M, Rodien P, Devor EJ, et al. 2004b. Modulation of Ligand Selectivity Associated with Activation of the Transmembrane Region of the Human Follitropin Receptor. Mol. Endocrinol. 18:2061–2073; doi:10.1210/me.2004-0036.

Olsson MHM, Søndergaard CR, Rostkowski M, Jensen JH. 2011. PROPKA3: Consistent Treatment of Internal and Surface Residues in Empirical pKa Predictions. J. Chem. Theory Comput. 7:525–537; doi:10.1021/ct100578z.

Ouyang F, Perry MJ, Venners SA, Chen C, Wang B, Yang F, et al. 2005. Serum DDT, age at menarche, and abnormal menstrual cycle length. Occup. Environ. Med. 62:878–884; doi:10.1136/oem.2005.020248.

Peltoketo H, Strauss L, Karjalainen R, Zhang M, Stamp GW, Segaloff DL, et al. 2010. Female mice expressing constitutively active mutants of FSH receptor present with a phenotype of premature follicle depletion and estrogen excess. Endocrinology 151:1872–1883; doi:10.1210/en.2009-0966.

Rignell-Hydbom A, Lindh CH, Dillner J, Jönsson BAG, Rylander L. 2012. A Nested Case-Control Study of Intrauterine Exposure to Persistent Organochlorine

Pollutants and the Risk of Hypospadias. J.-M.A. Lobaccaroed. PLoS ONE 7:e44767; doi:10.1371/journal.pone.0044767.

Rosenkilde MM, Benned-Jensen T, Frimurer TM, Schwartz TW. 2010. The minor binding pocket: a major player in 7TM receptor activation. Trends Pharmacol. Sci. 31:567–574; doi:10.1016/j.tips.2010.08.006.

Rossi M, Dimida A, Dell'anno MT, Trincavelli ML, Agretti P, Giorgi F, et al. 2007. The thyroid disruptor 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane appears to be an uncompetitive inverse agonist for the thyrotropin receptor. J. Pharmacol. Exp. Ther. 320:465–474; doi:10.1124/jpet.106.113613.

Sali A, Blundell TL. 1993. Comparative protein modelling by satisfaction of spatial restraints. J. Mol. Biol. 234:779–815; doi:10.1006/jmbi.1993.1626.

Saoudi A, Fréry N, Zeghnoun A, Bidondo M-L, Deschamps V, Göen T, et al. 2014. Serum levels of organochlorine pesticides in the French adult population: the French National Nutrition and Health Study (ENNS), 2006-2007. Sci. Total Environ. 472:1089–1099; doi:10.1016/j.scitotenv.2013.11.044.

Schug TT, Janesick A, Blumberg B, Heindel JJ. 2011. Endocrine disrupting chemicals and disease susceptibility. J. Steroid Biochem. Mol. Biol. 127:204–215; doi:10.1016/j.jsbmb.2011.08.007.

Sealfon SC, Chi L, Ebersole BJ, Rodic V, Zhang D, Ballesteros JA, et al. 1995. Related contribution of specific helix 2 and 7 residues to conformational activation of the serotonin 5-HT2A receptor. J. Biol. Chem. 270: 16683–16688.

Sherman W, Beard HS, Farid R. 2006a. Use of an induced fit receptor structure in virtual screening. Chem. Biol. Drug Des. 67:83–84; doi:10.1111/j.1747-0285.2005.00327.x.

Sherman W, Day T, Jacobson MP, Friesner RA, Farid R. 2006b. Novel procedure for modeling ligand/receptor induced fit effects. J. Med. Chem. 49:534–553; doi:10.1021/jm050540c.

Siegel ET, Kim H-G, Nishimoto HK, Layman LC. 2013. The Molecular Basis of Impaired Follicle-Stimulating Hormone Action: Evidence From Human Mutations and Mouse Models. Reprod. Sci. 20:211–233; doi:10.1177/1933719112461184.

Smits G, Olatunbosun O, Delbaere A, Pierson R, Vassart G, Costagliola S. 2003. Ovarian hyperstimulation syndrome due to a mutation in the follicle-stimulating hormone receptor. N. Engl. J. Med. 349: 760–766.

Sriraman V, Denis D, de Matos D, Yu H, Palmer S, Nataraja S. 2014. Investigation of a thiazolidinone derivative as an allosteric modulator of follicle stimulating

hormone receptor: Evidence for its ability to support follicular development and ovulation. Biochem. Pharmacol. 89:266–275; doi:10.1016/j.bcp.2014.02.023.

Strong AL, Shi Z, Strong MJ, Miller DF, Rusch DB, Buechlein AM, et al. 2014. Effects of the Endocrine-Disrupting Chemical DDT on Self-Renewal and Differentiation of Human Mesenchymal Stem Cells. Environ. Health Perspect.; doi:10.1289/ehp.1408188.

Sudharshan S, Naidu R, Mallavarapu M, Bolan N. 2012. DDT remediation in contaminated soils: a review of recent studies. Biodegradation 23:851–863; doi:10.1007/s10532-012-9575-4.

Ulloa-Aguirre A, Reiter E, Bousfield G, Dias JA, Huhtaniemi I. 2014. Constitutive activity in gonadotropin receptors. Adv. Pharmacol. San Diego Calif 70:37–80; doi:10.1016/B978-0-12-417197-8.00002-X.

Ulloa-Aguirre A, Zariñán T, Pasapera AM, Casas-González P, Dias JA. 2007. Multiple facets of follicle-stimulating hormone receptor function. Endocrine 32:251–263; doi:10.1007/s12020-008-9041-6.

Van Koppen CJ, Verbost PM, van de Lagemaat R, Karstens W-JF, Loozen HJJ, van Achterberg TAE, et al. 2013. Signaling of an allosteric, nanomolar potent, low molecular weight agonist for the follicle-stimulating hormone receptor. Biochem. Pharmacol. 85:1162–1170; doi:10.1016/j.bcp.2013.02.001.

Vasiliu O, Muttineni J, Karmaus W. 2004. In utero exposure to organochlorines and age at menarche. Hum. Reprod. Oxf. Engl. 19:1506–1512; doi:10.1093/humrep/deh292.

Vassart G, Pardo L, Costagliola S. 2004. A molecular dissection of the glycoprotein hormone receptors. Trends Biochem. Sci. 29:119–126; doi:10.1016/j.tibs.2004.01.006.

Vasseur C, Rodien P, Beau I, Desroches A, Gérard C, de Poncheville L, et al. 2003. A chorionic gonadotropin–sensitive mutation in the follicle-stimulating hormone receptor as a cause of familial gestational spontaneous ovarian hyperstimulation syndrome. N. Engl. J. Med. 349: 753–759.

Venners SA, Korrick S, Xu X, Chen C, Guang W, Huang A, et al. 2005. Preconception serum DDT and pregnancy loss: a prospective study using a biomarker of pregnancy. Am. J. Epidemiol. 162:709–716; doi:10.1093/aje/kwi275.

Vezzi V, Onaran HO, Molinari P, Guerrini R, Balboni G, Calò G, et al. 2013. Ligands raise the constraint that limits constitutive activation in G protein-coupled opioid receptors. J. Biol. Chem. 288:23964–23978; doi:10.1074/jbc.M113.474452.

Wang H, Li J, Gao Y, Xu Y, Pan Y, Tsuji I, et al. 2010. Xeno-oestrogens and phyto-oestrogens are alternative ligands for the androgen receptor. Asian J. Androl. 12:535–547; doi:10.1038/aja.2010.14.

Windham GC, Lee D, Mitchell P, Anderson M, Petreas M, Lasley B. 2005. Exposure to organochlorine compounds and effects on ovarian function. Epidemiol. Camb. Mass 16: 182–190.

Wohlfahrt-Veje C, Main KM, Skakkebæk NE. 2009. Testicular dysgenesis syndrome: foetal origin of adult reproductive problems. Clin. Endocrinol. (Oxf.) 71:459–465; doi:10.1111/j.1365-2265.2009.03545.x.

Wrobel J, Jetter J, Kao W, Rogers J, Di L, Chi J, et al. 2006. 5-Alkylated thiazolidinones as follicle-stimulating hormone (FSH) receptor agonists. Bioorg. Med. Chem. 14:5729–5741; doi:10.1016/j.bmc.2006.04.012.

Yanofsky SD, Shen ES, Holden F, Whitehorn E, Aguilar B, Tate E, et al. 2006. Allosteric Activation of the Follicle-stimulating Hormone (FSH) Receptor by Selective, Nonpeptide Agonists. J. Biol. Chem. 281:13226–13233; doi:10.1074/jbc.M600601200.

Younglai EV. 2004. Synergistic effects between FSH and 1,1-dichloro-2,2-bis(P-chlorophenyl)ethylene (P,P'-DDE) on human granulosa cell aromatase activity. Hum. Reprod. 19:1089–1093; doi:10.1093/humrep/deh252.

Yu HN, Richardson TE, Nataraja S, Fischer DJ, Sriraman V, Jiang X, et al. 2014. Discovery of substituted benzamides as follicle stimulating hormone receptor allosteric modulators. Bioorg. Med. Chem. Lett. 24:2168–2172; doi:10.1016/j.bmcl.2014.03.018.

Zoeller RT, Brown TR, Doan LL, Gore AC, Skakkebaek NE, Soto AM, et al. 2012. Endocrine-Disrupting Chemicals and Public Health Protection: A Statement of Principles from The Endocrine Society. Endocrinology 153:4097–4110; doi:10.1210/en.2012-1422.

Figure Legends

Figure 1. Effect of p,p'DDT on FSH-stimulated cAMP production. A) CHO-FSHR cells were incubated with hFSH at 3.10^{-2}UI/mL and 3 UI/mL and increasing concentrations of p,p'DDT were tested on. (means ± SEM of four independent experiments performed in triplicate) The cAMP concentration measured in presence of hFSH alone is arbitrarily set at 100%. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$, ****, $p<0.0001$ for the response in p,p'DDT-exposed compared with –unexposed cells, Mann-Whitney U test. B) Dose-response curve of hFSH on CHO-FSHR cells with or without p,p'DDT (5.10^{-6}M). (means ± SEM of eight independent experiments performed in triplicate) of the maximal response to FSH is arbitrarily set at 100%. ***, $p<0.001$, for the response in p,p'DDT-exposed compared with –unexposed cells, Two-way ANOVA test. C) Basal cAMP production of CHO-FSHR and CHO treated with p,p'DDT (5.10^{-6}M). (means ± SEM of four independent experiments performed in triplicate) The basal cAMP level in absence of p,p'DDT is arbitrarily set at 1. D) Cells were stimulated with 3UI/mL hFSH in the presence of p,p'DDT (5.10^{-6}M). The luminescence was recorded every minute. (means ± SEM of five independent experiments performed in triplicate) The maximal response of FSH is arbitrarily set at 100%. For clarity, the curve depicting the early phase of the kinetic is enlarged on the right.

Figure 2. Effect of p,p'DDT on calcitonin (A), hCG (B), and forskolin (FSK) (C) –stimulated cAMP production and on inhibition of PDE by IBMX (D). A) CHO-FSHR cells were stimulated for 30 min with increasing concentrations of salmon calcitonin (sCT) with or without of 5.10^{-6}M p,p'DDT (means ± SEM of three independent experiments performed in triplicate) the maximal response to sCT alone is arbitrarily set at 100. B) Basal and hCG stimulated (hCG 10^{-2} UI/mL and 100 UI/mL) cAMP production was measured in CHO-LH/hCGR cells, with or without p,p'DDT (means ± SEM of three independent experiments performed in triplicate). The cAMP production in absence of p,p'DDT is arbitrarily set at 100. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$, for the response in p,p'DDT-exposed compared with –unexposed cells, Mann-Whitney U test. C) CHO-FSHR and CHO cells were stimulated with forskolin 10^{-5}M (an adenylate cyclase (AC) agonist) with increasing dose of p,p'DDT (means ± SEM of three

independent experiments performed in triplicate) The cAMP production in presence of forskolin alone is arbitrarily set at 100. ***, p<0.001,****, p<0.0001, for the response in p,p'DDT-exposed compared with –unexposed cells, Mann-Whitney U test. D) CHO-FSHR cells were incubated with or without 1mM IBMX for 2 hours, then stimulated or not with FSH 3UI/mL with or without p,p'DDT 5.10^{-6}M. (means ± SEM of three independent experiments performed in triplicate). The cAMP production in presence of FSH alone is arbitrarily set at 1. *, p<0.05, **, p<0.01, for the response in p,p'DDT-exposed compared with –unexposed cells, Mann-Whitney U test.

Figure 3. The p,p'DDT targets the FSHR transmembrane domain. A) CHO-FSHR cell line was stimulated for 30 min by increasing doses of 16a in the presence of increasing p,p'DDT concentrations. (means ± SEM of six independent experiments performed in triplicate) The maximal response to 16a is arbitrarily set at 100. ***, p<0.001,****, p<0.0001, for the response in p,p'DDT-exposed compared with –unexposed cells, Mann-Whitney U test. B- F) Effect of p,p'DDT on mutant FSHR T3.32A (B), T3.32I (C), H7.42A (D),T3.32I-H7.42A (E) and rat FSHR (F) transiently expressed in CHO cells and stimulated for 30 min with increasing FSH concentrations with or without p,p'DDT (means ± SEM of three independent experiments performed in triplicate) and of the maximal response to hFSH in absence of p,p'DDT is arbitrarily set at 100. The basal activity measured in absence of FSH with (open columns) or without (filled columns). The basal activity in absence of p,p'DDT is arbitrarily set at 1. *, p<0.05, **, p<0.01, ****, p<0.0001, for the response in p,p'DDT-exposed compared with –unexposed cells, Mann-Whitney U test. G) Side and top views of the putative binding mode of p,p'DDT and 16a in the TMD of FSHR. p,p'DDT is shown as spheres (white star, C: slate; Cl: green). 16a is shown as sticks (C: white, N: blue, O: red). FSHR is shown with a ribbon representation. The helices are colored from blue for TM1 to red for TM7 and the intracellular TM8. Thr3.32 and His7.42, at the interface between the minor binding site (TM1-3,7) and the major binding site (TM3-7) are shown as spheres (black arrowhead, C: white, N:blue, O: red). p,p'DDT was docked to the minor binding pocket and the best pose was used for subsequent docking of 16a in the major binding pocket as described in Materials and Methods.

Figure 4. Effect of p,p'DDT on hCG and rhTSH – stimulated cAMP production in CHO-FSHR. CHO-FSHR cell line was stimulated for 30 min with increasing hCG or rhTSH concentrations with or without 5.10^{-6}M p,p'DDT. (means ± SEM of three independent experiments performed in triplicate) The maximal response to hCG or rhTSH is arbitrarily set at 100. **, $p<0.01$, ****, $p<0.0001$, for the response in p,p'DDT-exposed compared with –unexposed cells, Mann-Whitney U test.

Figure 5. Effect of p,p'DDE (A), o,p'DDT (B) and BPA (C) on FSH-stimulated cAMP production. CHO-FSHR cells were stimulated with hFSH 3.10^{-2}UI/mL (left) and 3 UI/mL (right) in presence of increasing doses of p,p'DDE (A), o,p'DDT (B) or BPA (C) (means ± SEM of three independent experiments performed in triplicate). The response to hFSH alone is arbitrarily set at 1. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$, ****, $p<0.0001$, for the response in p,p'DDE or o,p'DDT or BPA-exposed compared with – unexposed cells, Mann-Whitney U test.

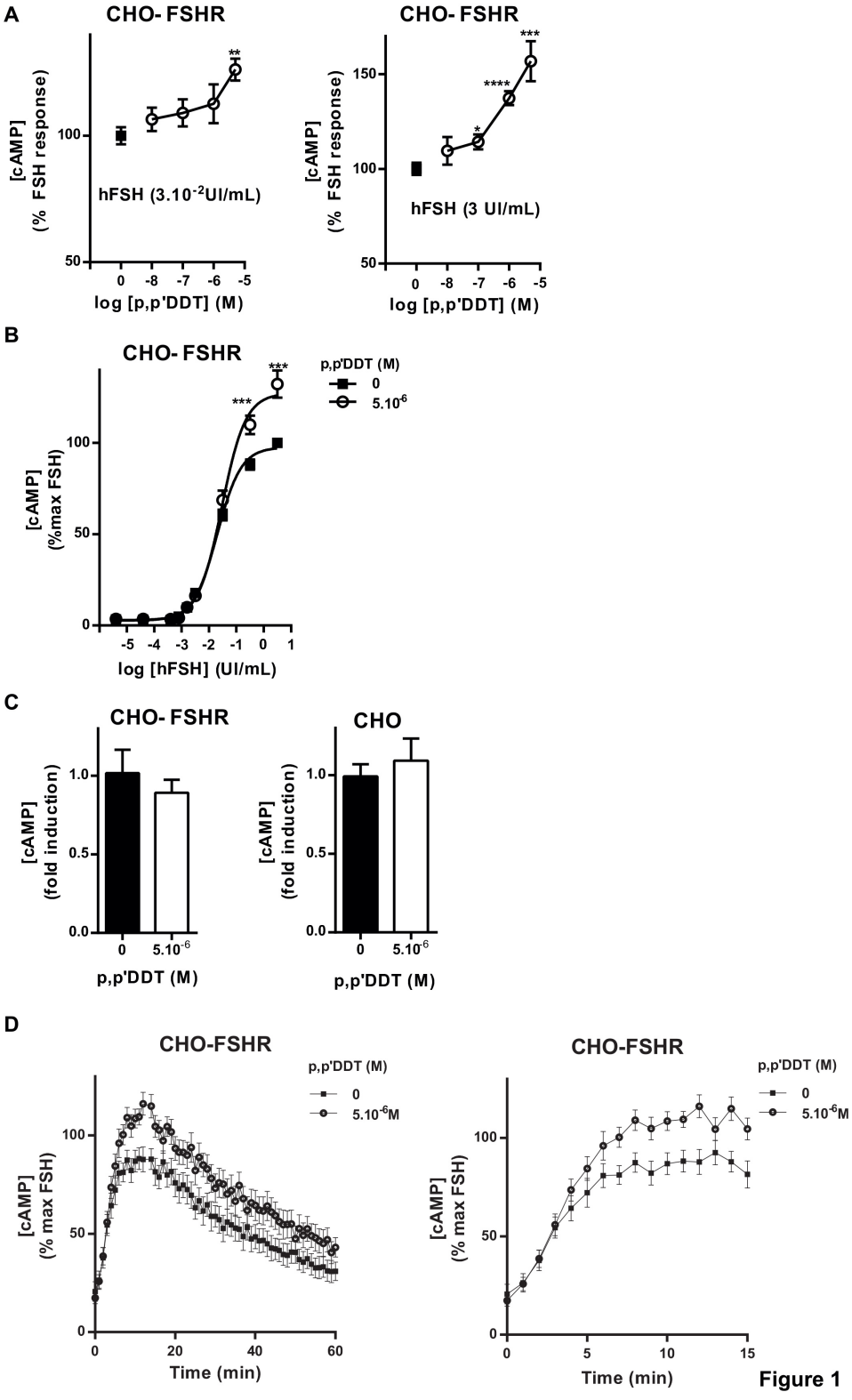
A
CHO- FSHR
[cAMP] (% FSH response)
hFSH (3.10-2UI/mL)
log [p,p'DDT] (M)
CHO- FSHR
hFSH (3 UI/mL)
log [p,p'DDT] (M)
B
CHO- FSHR
p,p'DDT (M)
0
5.10-6
[cAMP] (%max FSH)
log [hFSH] (UI/mL)
C
CHO- FSHR
CHO
[cAMP] (fold induction)
p,p'DDT (M)
D
CHO-FSHR
p,p'DDT (M)
0
5.10-6M
[cAMP] (% max FSH)
Time (min)

Figure 1

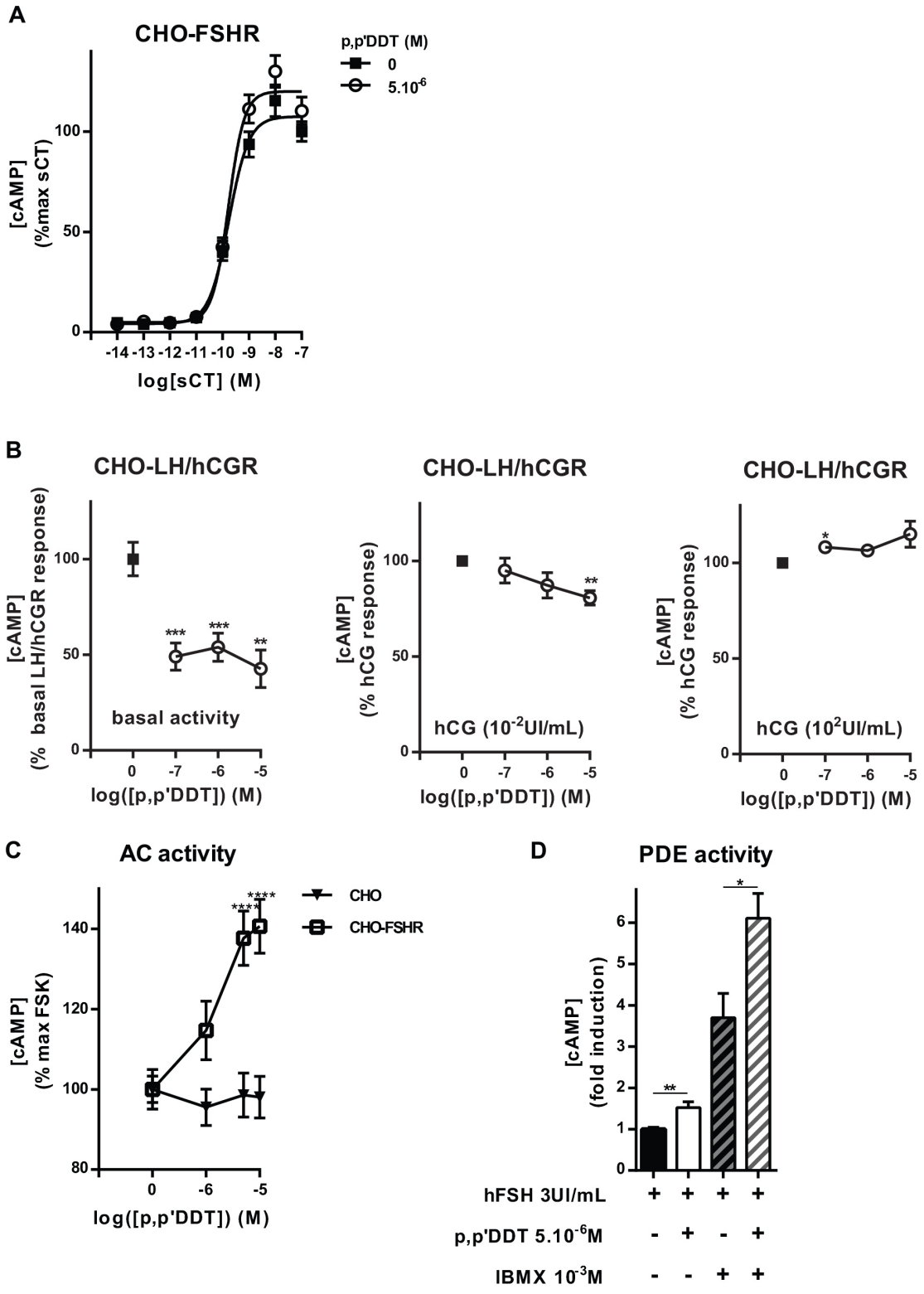
A
CHO-FSHR
p,p'DDT (M)
0
5.10-6
[cAMP] (%max sCT)
log[sCT] (M)
B
CHO-LH/hCGR
[cAMP] (% basal LH/hCGR response)
basal activity
log([p,p'DDT]) (M)
CHO-LH/hCGR
[cAMP] (% hCG response)
hCG (10-2UI/mL)
log([p,p'DDT]) (M)
CHO-LH/hCGR
[cAMP] (% hCG response)
hCG (102UI/mL)
log([p,p'DDT]) (M)
C
AC activity
CHO
CHO-FSHR
[cAMP] (% max FSK)
log([p,p'DDT]) (M)
D
PDE activity
[cAMP] (fold induction)
hFSH 3UI/mL
p,p'DDT 5.10-6M
IBMX 10-3M

Figure 2

Environ Health Perspect DOI: 10.1289/ehp.1510006
Advance Publication: Not Copyedited

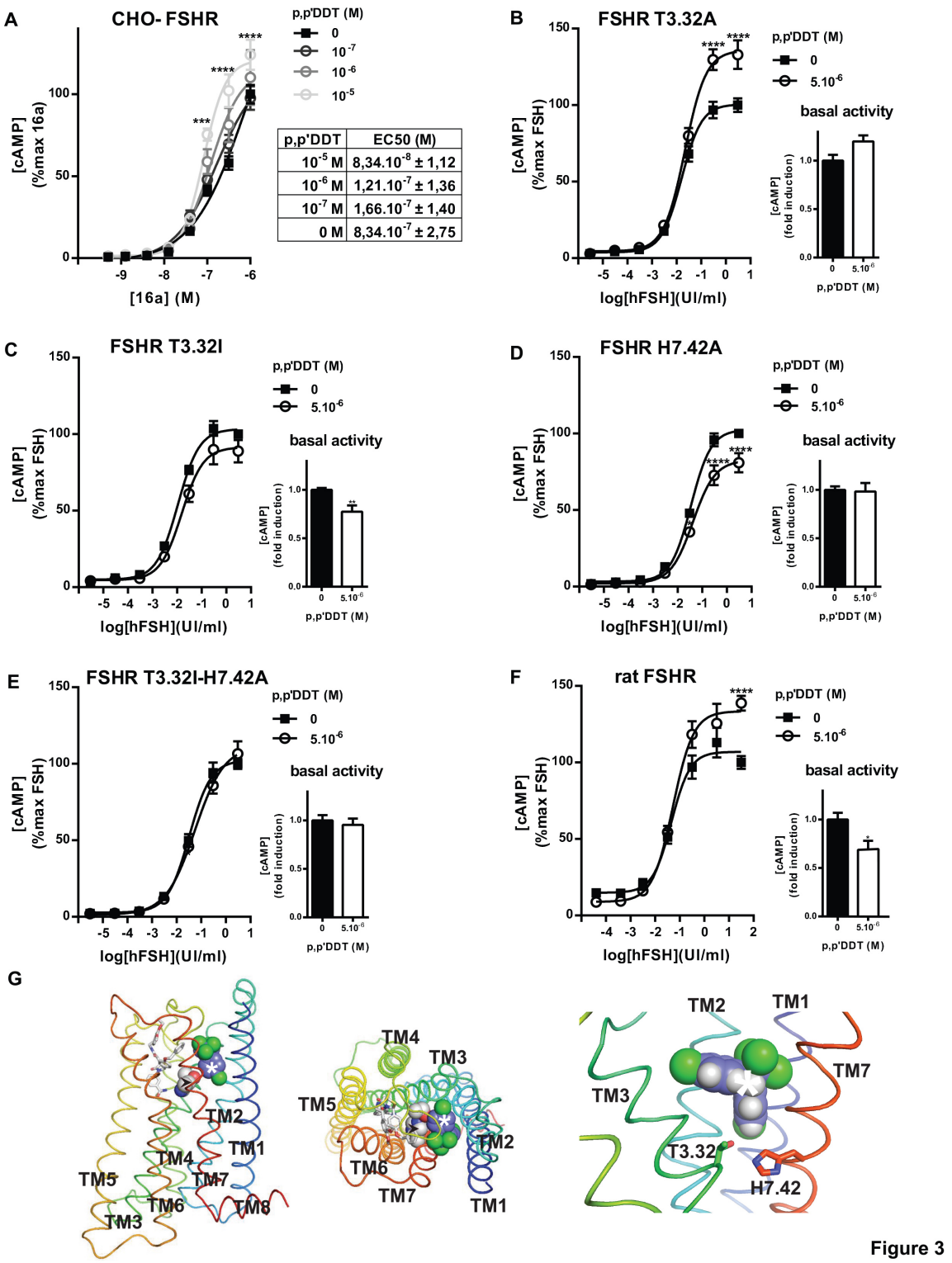

Figure 3

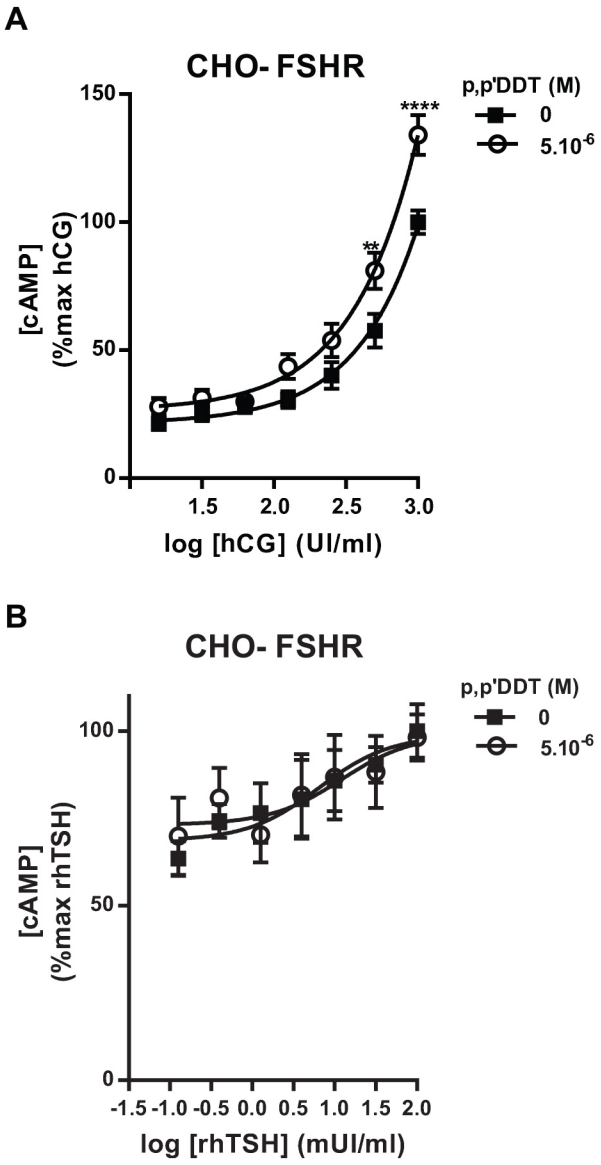
A
CHO- FSHR
p,p'DDT (M)
0
5.10-6
****
**
[cAMP]
(%max hCG)
150
100
50
0
1.5
2.0
2.5
3.0
log [hCG] (UI/ml)
B
CHO- FSHR
p,p'DDT (M)
0
5.10-6
[cAMP]
(%max rhTSH)
100
50
0
-1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0
log [rhTSH] (mUI/ml)

Figure 4

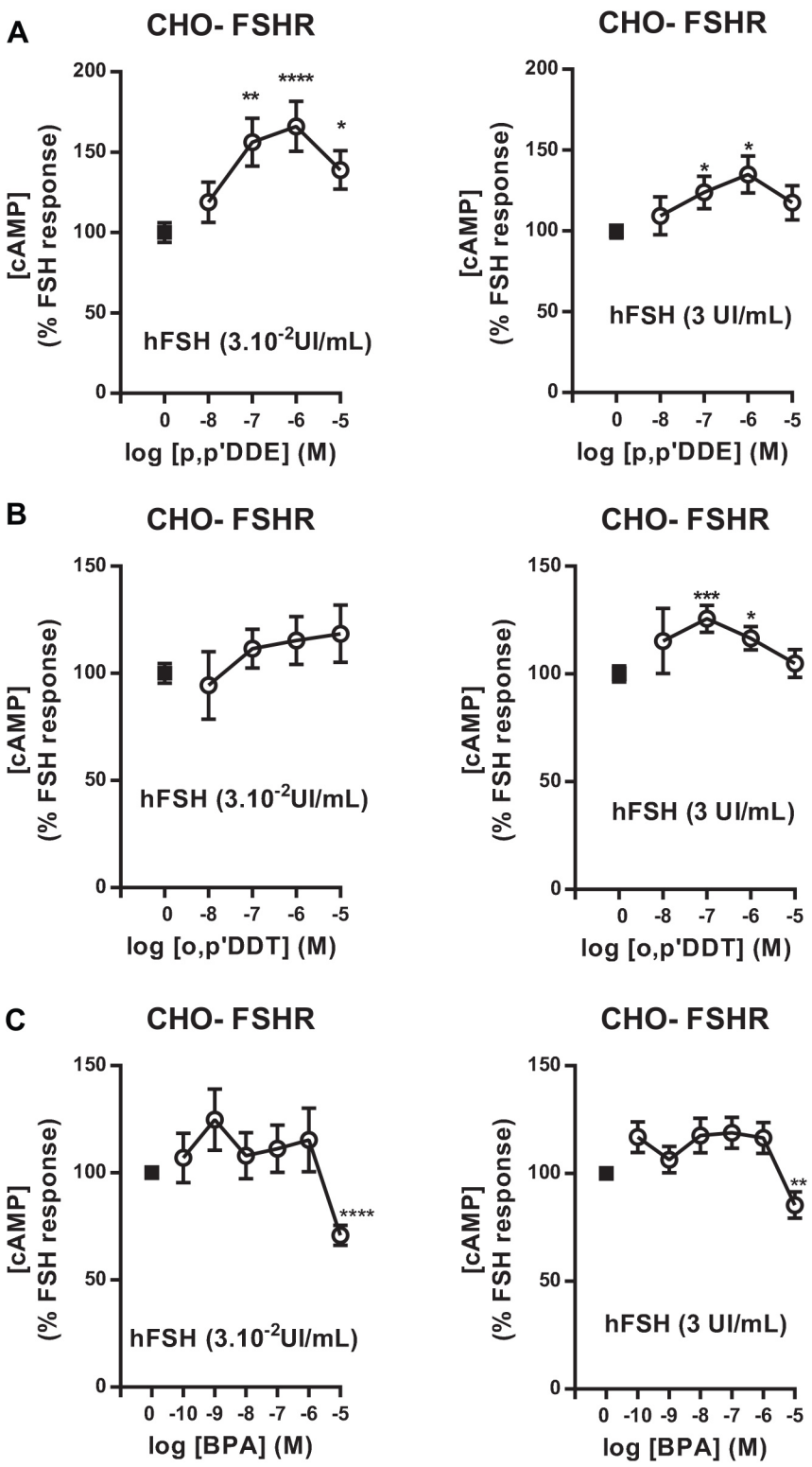
A
CHO- FSHR
[cAMP] (% FSH response)
hFSH (3.10-2UI/mL)
log [p,p'DDE] (M)
CHO- FSHR
hFSH (3 UI/mL)
log [p,p'DDE] (M)
B
CHO- FSHR
hFSH (3.10-2UI/mL)
log [o,p'DDT] (M)
CHO- FSHR
hFSH (3 UI/mL)
log [o,p'DDT] (M)
C
CHO- FSHR
hFSH (3.10-2UI/mL)
log [BPA] (M)
CHO- FSHR
hFSH (3 UI/mL)
log [BPA] (M)

Figure 5